

WHAT IS CLAIMED IS:

1. A method of analyzing a binding affinity of a receptor for a ligand in a plurality of mixtures comprising a receptor, E, a ligand, S_i , and a receptor-ligand binding pair, ES_i , the method comprising;

- 5 (a) providing a plurality of mixtures, each mixture comprising a receptor, $[E]_0$, a ligand $[S_i]_0$, and a titrant, T, wherein the concentration of one or more of E, S_i , T are chosen such that the relative ability of T to displace S can be determined;
- (b) allowing each of the plurality of mixtures to achieve equilibrium;
- (c) separating the receptor-ligand binding pairs, ES_i , from the unbound
10 ligands, S_i , for each of the plurality of mixtures;
- (d) determining the signal response from an analytical device for the receptor-ligand binding pair, ES_i in each of the plurality of mixtures; and
- (e) evaluating the signal responses from step (d) of the receptor-ligand binding pairs, ES_i , to determine binding affinity of the ligand S_i to the receptor E.

15 2. The method of claim 1, wherein each mixture is selected such that the concentration of T relative to $[E]_0$ and $[S_i]_0$, is chosen to allow for comparison of the relative ability of S_i to displace T.

20 3. The method of claim 1, comprising providing a plurality of mixtures, each mixture comprising a initial concentration of receptor, $[E]_0$, an initial concentration of ligand $[S_i]_0$, and a known concentration of a titrant wherein $[E]_0$ and $[S_i]_0$ are constant throughout each of the plurality of mixtures, the $[S_i]_0$ is approximately the same within each of the plurality of mixtures, and the concentration of the titrant is varied within the
25 plurality of mixtures.

4. The method of claim 1, wherein the plurality of mixture each comprise a plurality of ligands S_i , and a plurality of receptor-ligand binding pairs, ES_i , and wherein

the signal response is determined for at least two of the receptor-ligand binding pairs, ES_i , and the relative binding of the receptor-ligand binding pairs, ES_i , is determined.

5 5. The method of claim 4, wherein at least about 90% of the plurality of ligands, S_i , have a unique molecular mass.

6. The method of claim 1, wherein each mixture is selected such that the concentration of T relative to $[E]_0$ and $[S_i]_0$, is chosen such that the binding affinity of a first ligand, S_1 , can be compared with the binding affinity of a second ligand, S_2 , to provide a measure of the relative binding affinity of S_1 for E and S_2 for E.

7. The method of claim 1, wherein the binding affinities are relative binding equilibrium constants, K_{dis} .

15 8. The method of claim 1, step (e) comprising calculating the ACE_{50} , which is the titrant concentration at which the signal response of a receptor-ligand pair reaches 50% of its value when the titrant concentration is 0.

9. The method of claim 8, wherein relative K_d s of a plurality of ligands are
20 determined such that the ligand with the lowest ACE_{50} value has the highest K_d of the
mixture of ligands, and the ligand with the highest ACE_{50} value has the lowest K_d of the
mixture of ligands.

10. The method of claim 1, step (e) comprising calculating the K_{di} of a
25 receptor-ligand binding pair, ES_i , in the plurality of mixtures by fitting the change in
concentration of the receptor-ligand binding pairs, $[ES_i]$, in each of the plurality of
mixtures as a function of the titrant concentration to the equation of formula (I) or an
equation derived from formula (I)

$$K_{di} = \frac{([E]_0 - \sum_i [ES_i])([S]_0 - [ES_i])}{[ES_i]}$$

formula (I).

11. The method of claim 10, wherein the relative $K_{d,i}$ s of a plurality of ligands S_i are determined.

12. The method of claim 1, wherein the initial concentration of receptor, $[E]_0$,
5 is known and the initial concentration of the ligand, $[S_i]_0$ is known.

13. The method of claim 1, wherein the concentration of the receptor, $[E]_0$, is greater than the sum total of the concentration of the ligands, $[S_i]_0$.

10 14. The method of claim 1, further determining the whether a ligand S_i binds to the receptor E bind in a competitive manner, an allosteric manner, or a non-competitive manner.

15 15. The method of claim 14, wherein if a receptor ligand-pair ES_i maintains a relatively constant signal response in each of the plurality of mixtures the ligand S_i binds to the receptor E in a non-competitive manner.

16. The method of claim 14, comprising determining the variation in the ratio of signal response of a receptor ligand pair ES_i to response of the receptor-titrant pair
20 versus the concentration of the titrant for each of the plurality of mixtures, wherein if the ratios for each of the plurality of mixtures have a linear relationship with the titrant concentration, then the ligand S_i binds to the receptor in a competitive manner, and wherein if the ratios for each of the plurality of mixtures have a non-linear relationship, than the ligand S_i binds to the receptor in an allosteric manner.

25 17. The method of claim 1, wherein the receptor is a biomolecule.

18. The method of claim 1, wherein the receptor is a polypeptide.

30 19. The method of claim 1, wherein the receptor is an enzyme.

20. The method of claim 1, wherein the receptor is a nucleic acid.
21. The method of claim 1, wherein the ligand is an organic molecule.
- 5 22. The method of claim 1, wherein the ligand is a polypeptide.
23. The method of claim 1, wherein the plurality of mixtures achieves equilibrium of receptor-ligand binding pair, ES_i , unbound receptor, and unbound ligand.
- 10 24. The method of claim 1, further comprising using liquid chromatography.
25. The method of claim 1, wherein the receptor-bound ligand is separated from each of the plurality of mixtures using size-exclusion-chromatography.
- 15 26. The method of claim 1, wherein the receptor-bound ligand is separated from each of the plurality of mixtures using ultrafiltration.
27. The method of claim 1, wherein the signal response is determined using mass spectrometry.
- 20 28. The method of claim 1, further comprising disrupting the receptor-ligand binding pair, ES_i .
29. The method of claim 1, wherein the signal response of a receptor-ligand binding pair, ES_i , is determined by measuring the relative amount of ligand, S_i , in the receptor-ligand binding pair, ES_i , in each of the plurality of mixtures.
- 25 30. The method of claim 1, wherein the relative amount of ligand, S_i , is determined by evaluating a signal response from a mass spectrometer.
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31. A method for determining the equilibrium dissociation constant, K_d , of a receptor-ligand binding pair, the method comprising;

(a) providing a mass spectrometer calibrated to the ligand of the receptor-ligand binding pair;

5 (b) providing a plurality of mixtures, each mixture including a receptor, $[E]_0$, and a ligand, $[S]_0$, wherein the concentration of one or more of E_0 , and S_0 is chosen such that the binding affinity of S to E can be determined;

(c) allowing each of the plurality of mixtures to reach equilibrium of bound receptor-ligand binding pairs, ES, unbound receptor, and unbound ligand;

10 (d) separating the receptor-bound ligand from each of the plurality of mixtures;

(e) determining the signal response from the mass spectrometer for the receptor-ligand binding pairs in each of the plurality of mixtures; and

15 (f) using information known, measured or acquired in steps a-e to fit the concentration of receptor-ligand pair, $[ES]$, and initial, known ligand concentration, $[S]_0$, to the equation of formula (I)

$$K_d = \frac{([E]_0 - [ES])([S]_0 - [ES])}{[ES]}$$

formula (I)

20 for each of the plurality of mixtures, yielding the K_d of the receptor-ligand binding pair.

32. The method of claim 31, wherein each of the plurality of mixtures includes an initial concentration of receptor, $[E]_0$, and an initial, known concentration of ligand, $[S]_0$, wherein $[E]_0$ is about the same in each of the plurality of mixtures and $[S]_0$ is
25 varied in each of the plurality of mixtures.

33. The method of claim 31, further comprising determining the initial receptor concentration $[E]_0$ in the mixtures of step (b).

30 34. The method of claim 31, wherein the receptor is a biomolecule.

35. The method of claim 31, wherein the receptor is a polypeptide

36. The method of claim 31, wherein the receptor is an enzyme.

37. The method of claim 31, wherein the receptor is a nucleic acid.

38. The method of claim 31, wherein the ligand is an organic molecule.

39. The method of claim 31, wherein the ligand is a polypeptide.

40. The method of claim 31, wherein the plurality of mixtures reach equilibrium of bound receptor-ligand binding pairs, unbound receptor, and unbound ligand.

41. The method of claim 31, wherein the receptor-bound ligands are separated from the mixture using size-exclusion-chromatography.

42. The method of claim 31, further comprising using liquid chromatography.

43. The method of claim 31, further comprising disrupting the receptor-ligand binding pairs, ES.

44. The method of claim 31, wherein the concentration of the receptor-ligand binding pair, [ES], is determined in step (e) by measuring the amount of ligand in the receptor-ligand binding pairs, ES, in each of the plurality of mixtures.

45. A method of analyzing the binding kinetics of a receptor-ligand binding pair, the method comprising;

(a) providing a mixture comprising a receptor, $[E]_0$, and a ligand, $[S]_0$;

(b) allowing the mixture to reach equilibrium of receptor, [E], ligand, [S_i], and receptor-ligand binding pair, [ES_i];

(c) treating the mixture with an excess of a competitive inhibitor, I;

(d) measuring a decrease in the receptor-ligand binding pair at a plurality of time points by;

(i) separating the receptor-ligand binding pair from the unbound ligand; and

(ii) determining a signal response of the receptor-ligand binding pair for each of the plurality of time points with an analytical device; and

(e) using the information known, measured, or acquired from steps (a)-(d) to evaluate the binding kinetics of the receptor-ligand binding pair.

46. The method of claim 45, wherein the signal response of the receptor-ligand binding pair is measured with an analytical device.

47. The method of claim 45, wherein the mixture of step (a) comprises a plurality of ligands, S_i.

48. The method of claim 45, wherein at least 90% of the plurality of ligands, S_i, have a unique molecular mass.

49. The method of claim 45, wherein the binding kinetics are evaluated using the information known, measured, or acquired from steps (a)-(d) to calculate the dissociation rate, k_{s2} of the receptor-ligand binding pair by fitting the change in signal response of the receptor-ligand binding pair over time to the equation of formula (XVIII) or a derivative thereof

$$[ES] = [ES]_{t=0} e^{-k_{s2} \cdot t}$$

formula (XVIII).

50. The method of claim 45, comprising identifying a ligand that binds in a non-competitive manner wherein if the a ligand-receptor binding pair maintains a

relatively constant concentration at each of the plurality of time points, than the ligand is binding to the receptor in a non-competitive manner.

51. The method of claim 45, wherein the binding kinetics of at least two of the plurality of ligands, S_i , are compared.

52. The method of claim 45, wherein the receptor is a biomolecule.

53. The method of claim 45, wherein the receptor is a polypeptide.

54. The method of claim 45, wherein the receptor is an enzyme.

55. The method of claim 45, wherein the receptor is a nucleic acid.

56. The method of claim 45, wherein the ligand is an organic molecule.

57. The method of claim 45, wherein the ligand is a polypeptide.

58. The method of claim 45, wherein the competitive inhibitor is an organic molecule.

59. The method of claim 45, wherein the competitive inhibitor is a polypeptide.

60. The method of claim 45, further comprising subjecting the receptor-bound ligand to liquid chromatography.

61. The method of claim 45, wherein the receptor-bound ligand is separated from the unbound ligand using size-exclusion-chromatography.

62. The method of claim 45, wherein the signal response is determined using mass spectrometry.

5 63. The method of claim 45, further comprising disrupting the receptor-ligand binding pair.

64. The method of claim 45, wherein the signal response of the receptor-ligand binding pair is determined by measuring the relative amount of ligand in the receptor-ligand binding pair.

10 65. The method of claim 45, further comprising determining the half-life, $t_{1/2}$, of the receptor ligand binding pair.